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Synthesis and evaluation of some novel thiazoles and 1,3-thiazines as potent agents against the rabies virus

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Abstract: A series of novel thiazoles and 1,3-thiazine derivatives were synthesized in good yield via reaction of ethyl 3-(1-(2-thiocarbamoylhydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate with hydrazonoyl halides and aryliden-emalononitriles, respectively. The structure of the newly synthesized products was elucidated via elemental analysis, spectral data, and alternative routes whenever possible. Moreover, the antiviral screening of the products was evaluated and the results revealed that some of them have strong to moderate potency against the rabies virus compared with the reference drug.

Key words: Thiazoles, 1,3-thiazines, thiosemicarbazone, hydrazonoyl halides, antiviral evaluation

1. Introduction

Rabies is a worldwide zoonosis caused by a lyssavirus, involving many host species as reservoirs for infection. Developing countries in Africa and Asia still present endemic canine rabies and dogs remain the major animal reservoirs in such areas.[1] The rabies virus (RABV) is a negative stranded RNA virus belonging to the family Rhabdoviridae, genus Lyssavirus.[2] Pathogenetic mechanisms remain poorly understood, and treatment includes palliative measures only. Current medical emphasis relies heavily on prevention of exposure and intervention before clinical onset.[3] Once exposure occurs, modern prophylaxis entails immediate wound care, local infiltration of rabies immune globulin, and parenteral administration of rabies.

In 2005, the heroic recovery of an unvaccinated teenager from clinical rabies offered hope of future specific therapy. The treatment was based on an experimental approach with the employment of some drugs such as midazolam, ketamine, ribavirin, and amantadine.[4] In 2008, a Brazilian patient survived rabies with this experimental protocol and another successful recovery was reported in California in 2011.[5] Although few patients have survived rabies infection, currently there is no available treatment for this viral disease after the onset of clinical signs. In addition, this experimental treatment protocol has not been completely successful when applied to other patients. For this reason, the search for new antiviral agents against RABV is reinforced. Even though no significant efforts have been devoted to RABV-specific antiviral agents recently, interesting research has been performed in screening some in vitro antiviral agents against this virus. RABV infection was reduced when infected cells were treated with South American plants and algal polysaccharide extracts.[6,7] In addition, phenolic compounds were also tested and showed some activity against RABV.[8] In vitro anti-RABV activity is

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commonly assessed by inhibition of cytopathic effect (CPE).[6–8] Nevertheless, poor cytopathologic changes are observed in the most common cell lines employed, probably because of persistent infection without cellular lysis caused by the virus.[9] Previous reports[10–12] have demonstrated the presence of a visible and reliable CPE in McCoy cells infected with RABV. Inhibition of CPE might be time consuming when a large number of samples need to be analyzed. In vitro antiviral activity against many viruses is assessed by the MTT assay.[13] This is an established colorimetric method based on the determination of cell viability, as opposed to cell cytopathology with the use of a tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT) by the mitochondria of metabolically active cells. MTT is reduced by mitochondrial NADH and NADPH producing insoluble purple formazan crystals, which are later solubilized yielding a purple solution.[14] The color intensity of the final solution is directly proportional to cell viability and can be measured spectrophotometrically. This method is largely employed to determine the efficacy of antiviral compounds against the herpes virus (HSV),[15,16] human immunodeficiency virus (HIV),[17,18] influenza virus,[13,19] and many others. In the present study, we compared inhibition of the cytopathic effect and MTT assay in order to establish an alternative and accurate method for antirabies evaluation.

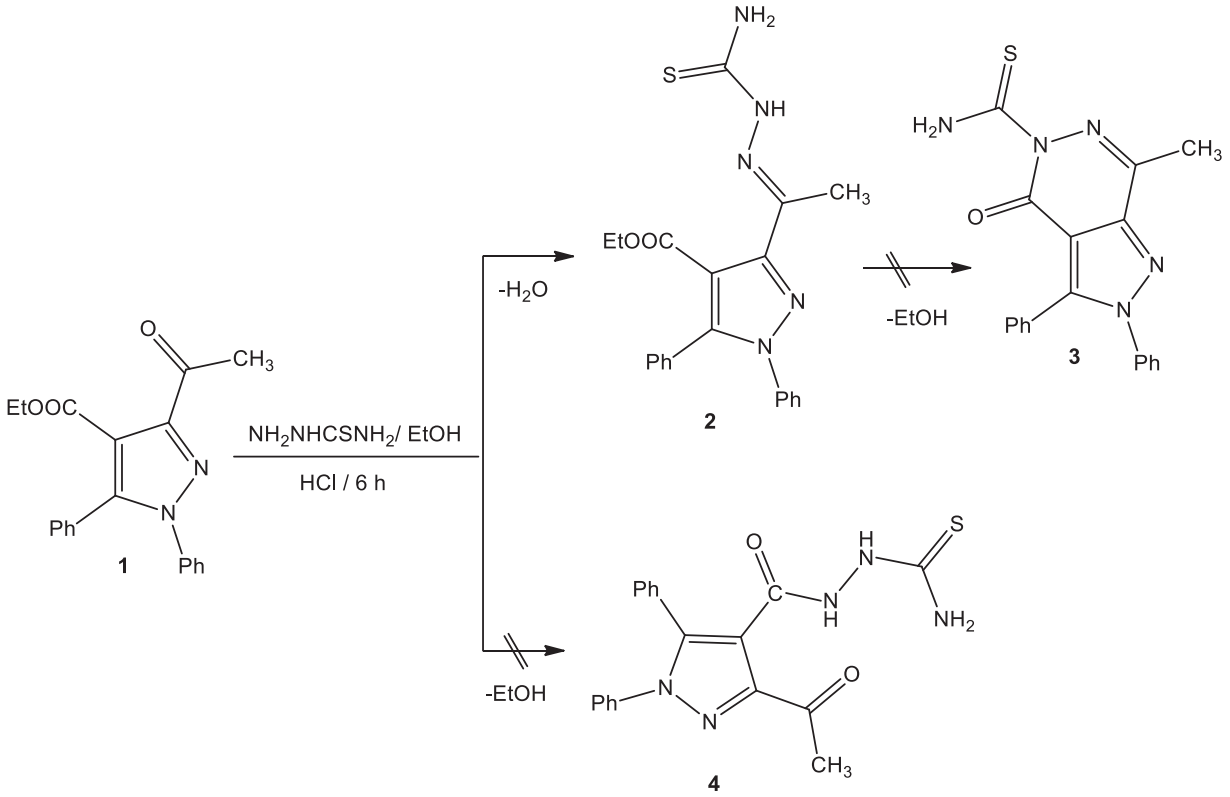
On the other hand, thiazole derivatives have attracted considerable interest owing to their wide spectra of biological activities such as antimicrobial, antioxidant, antitubercular, anticonvulsant, anticancer, and anti-inflammatory activity.[20–29] Moreover, 1,3-thiazine derivatives show a variety of biological potencies such as antimicrobial, anti-inflammatory, and anticancer.[29–33] These finding prompted us to report herein the synthesis of a new series of substituted thiazoles and 1,3-thiazines for testing their potency as antiviral agents.

2. Results and discussion

2.1. Chemistry

The new starting compound, namely ethyl 3-[1-(2-thiocarbamoylhydrazono)ethyl]-1,5-diphenyl-*1H*-pyrazole-4-carboxylate (**2**), was prepared by refluxing a mixture of ethyl 3-acetyl-1,5-diphenyl-*1H*-pyrazole-4-carboxylate (**1**)[34] and thiosemicarbazide in ethanol in the presence of a catalytic amount of hydrochloric acid for 6 h (Scheme 1). The structure of **2** was established based on elemental and spectral data (IR, ^{1}H NMR, mass). For example, the IR spectrum of compound **2** revealed stretching bands at υ = 3421 and 3160 cm^{-1}, assigned to the NH and NH_2 groups, in addition to another band at υ = 1692 cm^{-1}, attributed to the conjugated ester carbonyl group. The ^{1}H NMR spectrum of compound **2** displayed two signals at δ =3.17 and 10.69 ppm, attributed to the NH_2 and NH protons, in addition to the expected signals of the ester group, methyl, and aromatic protons. The mass spectrum revealed a molecular ion peak at m/z = 407, which is consistent with the molecular formula of compound **2** and not **3** or **4**.

Next our study was extended to investigate the reactivity of compound **2** towards hydrazonoyl halides, aiming to synthesize new heterocyclic compounds containing a 1,3-thiazole ring. Thus, reaction of compound **2** with N-aryl hydrazonoyl chloride (or bromide) **5** in dioxane under reflux in the presence of triethylamine as basic catalyst afforded one isolable product (as evidenced by TLC analysis of the crude product), which were identified to be products **7** (Scheme 2). The structure of products **7** was elucidated by elemental and spectral (IR, ^{1}H NMR, mass) data. The IR spectra of products **7** showed in each case two stretching bands at υ = 1692 and 3421–3160 cm^{-1}, assigned to the carbonyl and the NH groups. The ^{1}H NMR spectra of compounds **7** revealed in addition to the expected signals of the aromatic protons, and the protons of the substituted R group and the methyl group, a singlet signal at δ =10.69 ppm, assigned to the –NH proton. The mass spectra

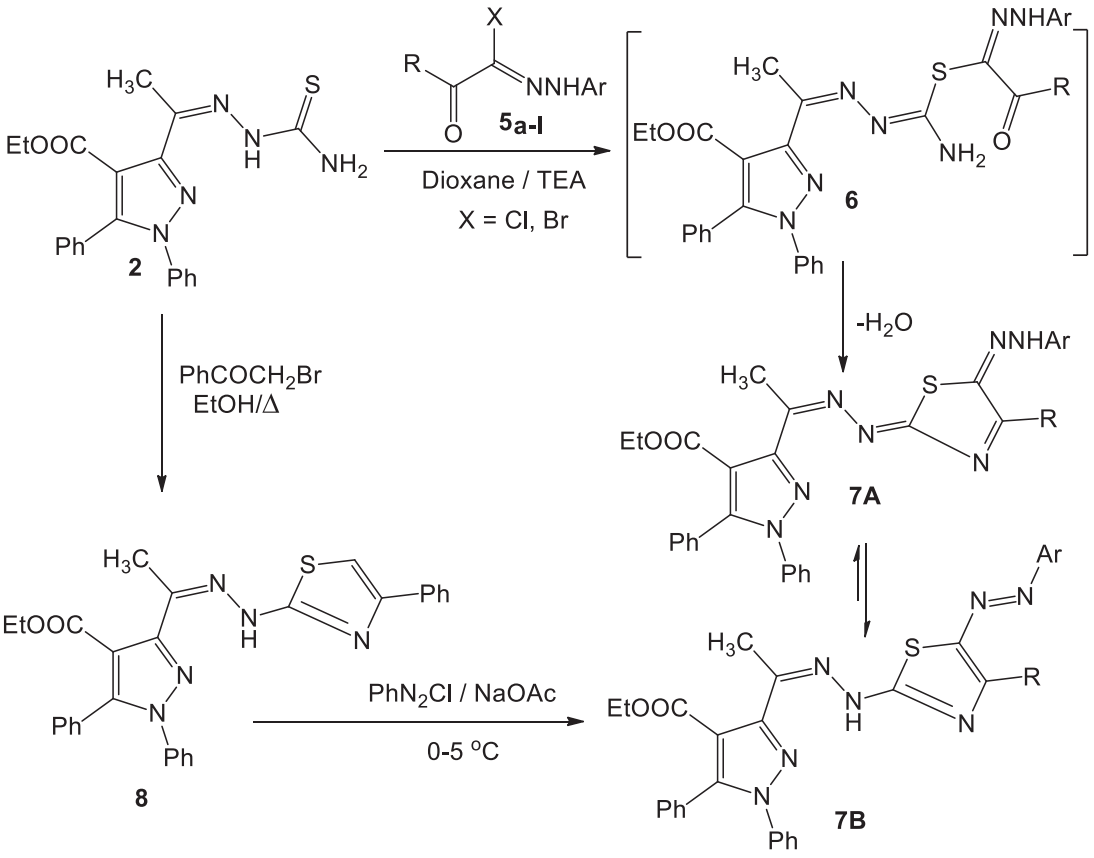


Scheme 1. Synthesis of thiosemicarbazone derivative **2**.

of all products **7** exhibited in each case a molecular ion peak at the correct molecular weight for the respective compound (see Experimental). To account for the formation of products **7**, it was suggested that intermediate **6** is initially formed via nucleophilic attack of the thiol group of compound **2** to the electron-deficient carbon of the hydrazone group of compound **5**, which undergoes dehydrative cyclization to give the final products **7**, which can exist in two possible tautomeric forms, **A** and/or **B**.

The structure of products **7** was further confirmed by an alternative method. Thus, reaction of compound **2** with phenacyl bromide under reflux in ethanol led to the formation of product **8**. Compound **8** was then reacted with benzenediazonium salt in ethanol in the presence of sodium acetate trihydrate at 0–5 °C to give a product identical in all respects (IR, mp, and mixed mp) with **7k**, which obtained from reaction of **2** with hydrazonoyl halide **5k** (Scheme 2).

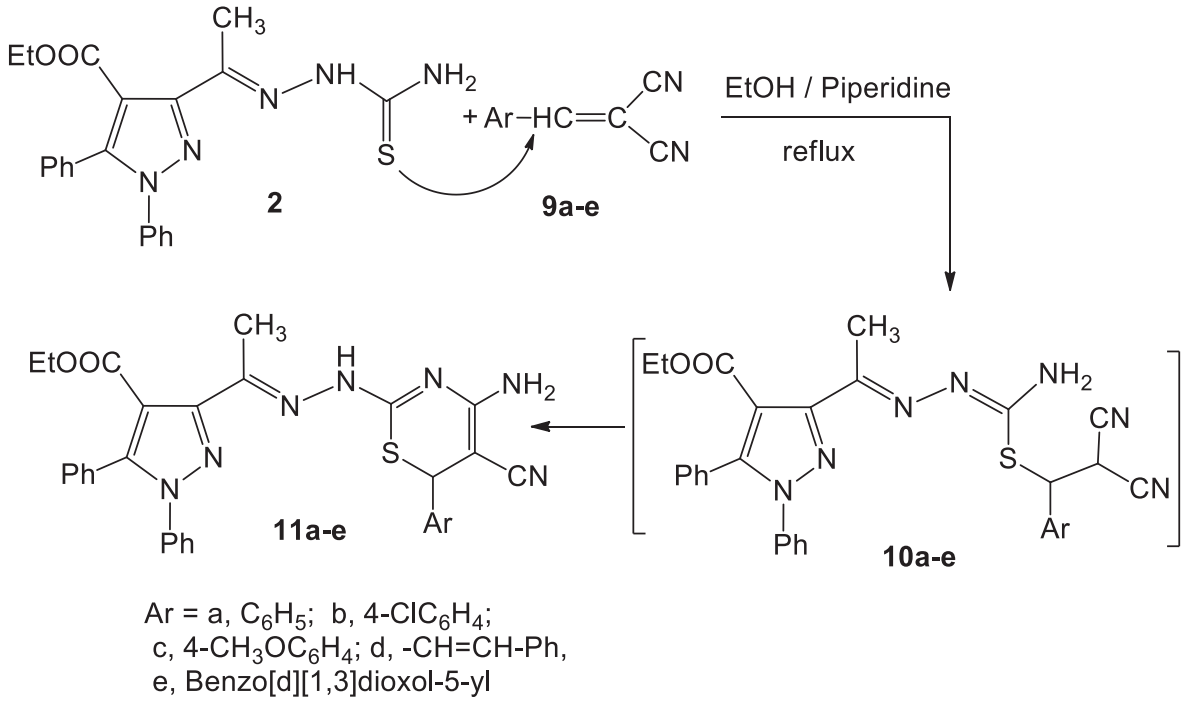
Furthermore, the utility of compound **2** as a building block for the synthesis of another series of expected biologically active heterocycles was explored through its reaction with arylidene malononitrile. Thus, reaction of compound **2** with the appropriate arylidene malononitrile in absolute ethanol under reflux and in the presence of a catalytic amount of piperidine afforded in each case only one isolable product (as evidenced by TLC analysis of the crude product) (Scheme 3). The latter products were identified to be **11** on the basis of elemental and spectral (IR, ^{1}H NMR, and mass) data. The IR spectra revealed in each case four bands at υ = 1691, 2187, 3423, and 3159 cm^{-1}, which are assigned to the ester carbonyl, nitrile, –NH and, –NH$_2$ groups. The ^{1}H NMR spectrum of product **11a**, taken as a representative example of the products **11**, revealed in addition to the expected signals assigned for the $COOC_2H_5$, CH_3, and aromatic protons two signals at δ = 8.40 and 10.68 ppm assigned for the NH_2 and NH protons.



5,7	R	Ar	5,7	R	Ar
a	CH_3	C_6H_5	**g**	CH_3	4-$NO_2C_6H_4$
b	CH_3	2-$CH_3C_6H_4$	**h**	CH_3	4-ClC_6H_4
c	CH_3	3-$CH_3C_6H_4$	**i**	CH_3	4-BrC_6H_4
d	CH_3	4-$CH_3C_6H_4$	**j**	CH_3	2,4-$(Cl_2)C_6H_3$
e	CH_3	4-$CH_3OC_6H_4$	**k**	C_6H_5	C_6H_5
f	CH_3	4-$CH_3COC_6H_4$	**l**	2-C_4H_3S	C_6H_5

Scheme 2. Synthesis of thiazoles **7a**–**l**.

In addition, the reaction of compound **2** with dimethyl acetylenedicarboxylate was also investigated, aiming to prepare new bioactive heterocyclic compounds. Thus, reaction of compound **2** with dimethyl acetylenedicarboxylate (DMAD) in methanol under reflux afforded product **13** via elimination of methanol from the nonisolable intermediate **12** (Scheme 4). The other isomeric structure **14** was excluded on the basis of ^{1}H NMR spectral data (IR and mass spectral data of **13** and **14** are nearly the same). For example, the ^{1}H NMR spectrum of product **13** revealed the presence of a singlet signal at $\delta = 6.65$ ppm assigned to the olefinic CH proton of the =CH–COOMe group, in addition to the signals of the aromatic, methyl, and ester protons (see Experimental).



Scheme 3. Synthesis of 1,3-thiazines **11a**–**e**.

2.2. Antiviral activity: antiviral testing of some new chemical derivatives in rabies

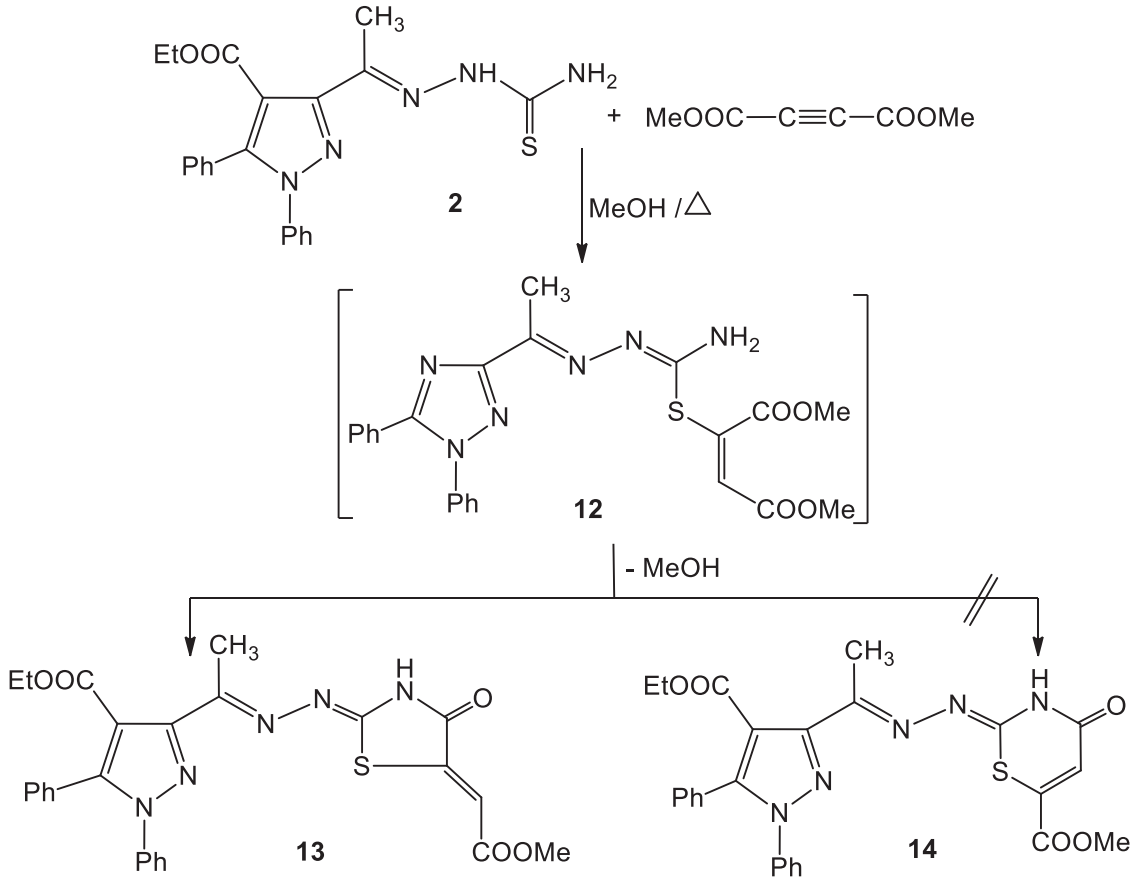
The newly synthesized compounds were tested for their cytotoxic activity using Vero-cell culture and their antiviral activity was tested against Herpes simplex virus type 1 (HSV-1) using the antiviral antimitotic antibiotic aphidicolin as a positive control.[37–39] The results of the cytotoxic and antiviral activity of the synthesized compounds and the antiviral antibiotic aphidicolin are shown in the Table.

The results revealed that compounds **7b**–**l**, **8**, **11a**, **11c**, **11e**, and **13** showed strong to moderate antiviral activity compound with the reference drug used as positive control. The tested compounds exhibited minimum antiviral concentration (mg/mL) as follows: **7e** (0.008), **7d** (0.01), **7b** (0.05), **7c** (0.08), and **7a** (0.09), which indicates that compound **7e** is the most effective one as an antiviral agent against the rabies virus. The rest of the tested compounds were found to be inactive.

3. Experimental section

3.1. Chemistry

Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded in a Pye-Unicam SP300 instrument in potassium bromide discs. ^{1}H and ^{13}C NMR spectra were recorded in a Varian Mercury VXR-300 spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) in DMSO-d_6 and the chemical shifts were related to that of the solvent. Mass spectra were recorded in a GCMS-QP 1000 EX Shimadzu Spectrometer; the ionizing voltage was 70 eV. Elemental analyses were carried out at the Microanalytical Laboratory of Cairo University, Giza, Egypt. Antiviral activity of the products was determined at the Veterinary and Serum Research Institute, Giza, Egypt. Hydrazonoyl halides **5a**–**l**[40–44] were previously reported.



Scheme 4. Synthesis of thiazolidinone derivative **13**.

Synthesis of ethyl 3-(1-(2-thiocarbamoylhydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (2). A solution of ethyl 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carboxylate **1** (3.34 g, 10 mmol) and thiosemicarbazide (0.91 g, 10 mmol) in ethanol (50 mL) containing a catalytic amount of hydrochloric acid was refluxed for 6 h. The reaction mixture was left to cool and the precipitate formed was filtered, washed with ethanol, and recrystallized from acetic acid to give pure product of compound **2** as yellowish-white solid (72%); mp = 220–222 °C; IR (KBr): ν =3421, 3275, 3160 (NH_2, NH), 1692 (C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-*d*6): δ1.09 (t, J = 7.2, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.17 (s, br, 2H, NH_2), 4.02 (q, J = 7.2, 2H, CH_2), 7.20–7.38 (m, 10H, Ar-H),10.69 (s, br, 1H, NH); MS m/z(%): 407 (M^+, 14), 392 (34), 346 (51), 180 (43), 77 (100). Anal. Calcd for $C_{21}H_{21}N_5O_2S$ (407.49): C, 61.90; H, 5.19; N, 17.19. Found C, 61.79; H, 5.10; N, 17.11%.

Synthesis of ethyl 3-(1-(2-(4-substituted-5-(arylazo)thiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7a–l).

To a solution of thiosemicarbazone **2** (0.407 g, 1 mmol) and the appropriate hydrazonoyl halides **5** (1 mmol) in hot dioxane (15 mL) was added triethylamine (0.14 mL). The reaction mixture was refluxed for 4–8 h, allowed to cool, and the solid formed was filtered off, washed with ethanol, dried, and recrystallized from ethanol to give the corresponding thiazoles **7a**–**l**. The physical constants with the spectral data of products **7a**–**l** are given below.

Table. The cytotoxic concentration (CD50) and the antiviral activity against HSV-1 of the synthesized compounds **7**, **8**, **11**, and **13**.

Compound no.	Cytotoxicity (CD50) mg/mL	Minimum antiviral conc. (mg/mL)
7a	0.02	0.09
7b	0.03	0.05
7c	0.03	0.08
7d	0.02	0.01
7e	0.02	0.008
7f	0.02	0.12
7g	0.01	0.11
7h	0.01	0.18
7i	0.02	0.13
7j	0.03	0.15
7k	0.01	0.14
7l	0.01	0.17
8	0.01	0.21
11a	0.02	0.27
11b	-	-
11c	0.01	0.20
11d	-	-
11e	0.02	021
13	0.02	0.22
Aphidicolin	0.20	0.005

Ethyl 3-(1-(2-(4-methyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7a). Red solid, (70% yield); mp 85–87 °C; IR (KBr) ν =3436 (NH), 1715 (C=O), 1599 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.08 (t, J = 7.2, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.16 (q, J = 7.2, 2H, CH_2), 6.98–7.43 (m, 15H, Ar-H), 10.61 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ11.4, 13.4, 16.8 (CH_3), 60.4 (CH_2), 114.5, 123.2, 125.4, 125.9, 127.3, 128.0, 128.3, 129.2, 129.6, 129.8, 130.3, 132.4, 134.7, 137.5, 140.2, 140.7, 144.5, 148.4, 154.8 (Ar-C), 164.6 (C=O); MS, m/z (%) 549 (M^+, 4), 503 (16), 301 (48), 180 (34), 77 (100). Anal. calcd for $C_{30}H_{27}N_7O_2S$ (549.65): C, 65.56; H, 4.95; N, 17.84. Found: C, 65.48; H, 4.82; N, 17.73%.

Ethyl 3-(1-(2-(4-methyl-5-(o-tolyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7b). Red solid, (72% yield); mp 84–86 °C; IR (KBr) ν =3433 (NH), 1719 (C=O), 1600 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.06 (t, J = 7.2, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.22 (q, J = 7.2, 2H, CH_2), 7.11–7.49 (m, 14H, Ar-H), 10.61 (s, br, 1H, NH); MS, m/z (%) 563 (M^+, 9), 517 (37), 272(24), 180 (54), 77 (100). Anal. calcd for $C_{31}H_{29}N_7O_2S$ (563.67): C, 66.05; H, 5.19; N, 17.39. Found: C, 66.02; H, 5.08; N, 17.32%.

Ethyl 3-(1-(2-(4-methyl-5-(m-tolyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7c). Red solid, (66% yield); mp 82–84 °C; IR (KBr) ν =3437 (NH), 1710 (C=O), 1602 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.04 (t, J = 7.2, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.22 (q, J = 7.2, 2H, CH_2), 7.17–7.39 (m, 14H, Ar-H),10.54 (s, br, 1H, NH);

MS, m/z (%) 563 (M^+, 25), 517 (36), 359 (35), 301 (25), 180 (58), 77 (100). Anal. calcd for $C_{31}H_{29}N_7O_2S$ (563.67): C, 66.05; H, 5.19; N, 17.39. Found: C, 66.12; H, 5.12; N, 17.26%.

Ethyl 3-(1-(2-(4-methyl-5-(p-tolyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7d). Dark red solid, (70% yield); mp 90–92 °C; IR (KBr) ν =3430 (NH), 1715 (C=O), 1603 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.06 (t, J = 7.2, 3H, CH_3), 2.21 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.17 (q, J = 7.2, 2H, CH_2), 7.12–7.40 (m, 14H, Ar-H), 10.60 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ11.4, 13.7, 16.4, 21.3 (CH_3), 60.5 (CH_2), 114.3, 125.6, 125.8, 125.9, 127.9, 128.2, 128.9, 129.2, 129.6, 129.8, 132..0, 132.2, 137.3, 139.6, 140.6, 142.5, 144.5, 147.4, 154.8 (Ar-C), 164.4 (C=O); MS, m/z (%) 563 (M^+, 8), 517 (37), 301 (42), 180 (59), 77 (100). Anal. calcd for $C_{31}H_{29}N_7O_2S$ (563.67): C, 66.05; H, 5.19; N, 17.39. Found: C, 66.18; H, 5.03; N, 17.25%.

Ethyl 3-(1-(2-(5-((4-methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono) ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7e). Orange solid, (73% yield); mp 76–78 °C; IR (KBr) ν =3432 (NH), 1705 (C=O), 1599 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.06 (t, J = 7.2, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.16 (q, J = 7.2, 2H, CH_2), 6.85–7.40 (m, 14H, Ar-H), 10.61 (s, br, 1H, NH); MS, m/z (%) 580 (M^+, 4), 563 (7), 359 (43), 180 (34), 77 (100). Anal. calcd for $C_{31}H_{29}N_7O_3S$ (579.67): C, 64.23; H, 5.04; N, 16.91. Found: C, 64.21; H, 5.02; N, 16.75%.

Ethyl 3-(1-(2-(5-((4-acetylphenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7f). Orange solid, (72% yield); mp 106–108 °C; IR (KBr) ν =3426 (NH), 1713, 1670 (2C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.04 (t, J = 7.2, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.62 (s, 3H,CH_3CO), 4.18 (q, J = 7.2, 2H, CH_2), 7.31–7.95 (m, 14H, Ar-H), 10.91 (s, br, 1H, NH); MS, m/z (%) 591 (M^+, 4), 528 (4), 301 (52), 219 (19), 120 (100), 64 (99). Anal. calcd for: $C_{32}H_{29}N_7O_3S$ (591.68): C, 64.96; H, 4.94; N, 16.57. Found: C, 64.91; H, 4.78; N, 16.46%.

Ethyl 3-(1-(2-(4-methyl-5-((4-nitrophenyl)diazenyl)thiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7g). Orange solid, (74% yield); mp 112–114 °C; IR (KBr) ν =3435 (NH), 1709 (C=O), 1594 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.19 (t, J = 7.2, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.18 (q, J = 7.2, 2H, CH_2), 7.32–8.27 (m, 14H, Ar-H), 10.72 (s, br, 1H, NH); MS, m/z (%) 594 (M^+, 7), 578 (3), 301 (51), 180 (39), 64 (100). Anal. calcd for $C_{30}H_{26}N_8O_4S$ (594.64): C, 60.59; H, 4.41; N, 18.84. Found: C, 60.45; H, 4.36; N, 18.72%.

Ethyl 3-(1-(2-(5-((4-chlorophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7h). Red solid, (69% yield); mp 88–90 °C; IR (KBr) ν =3428 (NH), 1711 (C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.04 (t, J = 7.2, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.18 (q, J = 7.2, 2H, CH_2), 7.22–7.61 (m, 14H, Ar-H), 10.64 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ11.6, 13.5, 16.4 (CH_3), 60.7 (CH_2), 114.9, 125.2, 125.8, 127.3, 127.9, 128.0, 128.7, 129.2, 129.6, 130.2, 131.2, 134.5, 137.3, 139.3, 140.0, 141.7, 142.4, 147.2, 156.7 (Ar-C), 164.8 (C=O); MS, m/z (%) 586 (M^++2, 1), 584 (M^+, 3), 537 (34), 301 (64), 180 (62), 77 (100). Anal. calcd for $C_{30}H_{26}ClN_7O_2S$ (584.09): C, 61.69; H, 4.49; N, 16.79. Found: C, 61.58; H, 4.41; N, 16.68%.

Ethyl 3-(1-(2-(5-((4-bromophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7i). Dark red solid, (70% yield); mp 86–88 °C; IR (KBr) ν =3426 (NH), 1713 (C=O), 1595 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.06 (t, J = 7.2, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.18 (q, J = 7.2, 2H, CH_2), 7.27–7.55 (m, 14H, Ar-H), 10.68 (s, br, 1H, NH);

MS, m/z (%) 628 (M^+, 2), 581 (5), 301 (58), 180 (24), 77 (100). Anal. calcd for $C_{30}H_{26}BrN_7O_2S$ (628.54): C, 57.33; H, 4.17; N, 15.60. Found: C, 57.33; H, 4.17; N, 15.60%.

Ethyl 3-(1-(2-(5-((2,4-dichlorophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono) ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7j). Dark red solid, (74% yield); mp 90–92 °C; IR (KBr) ν =3433 (NH), 1711 (C=O), 1589 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.20 (t, J = 7.2, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.24 (q, J = 7.2, 2H, CH_2), 7.17–7.73 (m, 13H, Ar-H),10.68 (s, br, 1H, NH); MS, m/z (%) 618 (M^+, 2), 571 (4), 536 (3), 301 (15), 180 (48), 77 (100). Anal. calcd for $C_{30}H_{25}Cl_2N_7O_2S$ (618.54): C, 58.25; H, 4.07; N, 15.85. Found: C, 58.16; H, 4.02; N, 15.72%.

Ethyl 1,5-diphenyl-3-(1-(2-(4-phenyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazono) ethyl)-1*H*-pyrazole-4-carboxylate (7k). Red solid, (70% yield); mp 96–98 °C; IR (KBr) ν =3423 (NH), 1693 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ0.99 (t, J = 7.2, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.04 (q, J = 7.2, 2H, CH_2), 7.21–7.39 (m, 20H, Ar-H), 10.69 (s, br, 1H, NH); MS, m/z (%) 611 (M^+, 2), 551 (4), 346 (43), 180 (35), 77 (100). Anal. calcd for $C_{35}H_{29}N_7O_2S$ (611.72): C, 68.72; H, 4.78; N, 16.03. Found: C, 68.65; H, 4.78; N, 15.92%.

Ethyl 1,5-diphenyl-3-(1-(2-(5-(phenyldiazenyl)-4-(2-thienyl)thiazol-2-yl)hydrazono) ethyl)-1*H*-pyrazole-4-carboxylate (7l). Dark red solid, (70% yield); mp 90–92 °C; IR (KBr) ν =3428 (NH), 1712 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.05 (t, J = 7.2, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.04 (q, J = 7.2, 2H, CH_2), 7.09–8.08 (m, 18H, Ar-H), 10.68 (s, br, 1H, NH); MS, m/z (%) 617 (M^+, 2), 527 (48), 272 (16), 180 (34), 77 (100). Anal. calcd for $C_{33}H_{27}N_7O_2S_2$ (617.74): C, 64.16; H, 4.41; N, 15.87. Found: C, 64.12; H, 4.36; N, 15.76%.

Synthesis of ethyl 1,5-diphenyl-3-(1-(2-(4-phenylthiazol-2-yl)hydrazono)ethyl)-1*H*-pyrazole-4-carboxylate (8)

A mixture of thiosemicarbazone **2** (0.407 g, 1 mmol) and phenacyl bromide (0.197 g, 1 mmol) in ethanol (10 mL) was refluxed for 2 h and then left to cool to room temperature. The precipitate formed was filtered off, washed with ethanol, and recrystallized from ethanol to give the thiazole derivative **8** as yellow solid, (69% yield); mp 244–246 °C; IR (KBr) ν =3433 (NH), 1699 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.06 (t, J = 7.2, 3H, CH_3), 2.49 (s, 3H, CH_3), 4.06 (q, J = 7.2, 2H, CH_2), 7.33–7.48 (m, 16H, Ar-H and thiazole-H5), 10.69 (s, br, 1H, NH); MS, m/z (%) 507 (M^+, 3), 461 (27), 272 (6), 180 (17), 113 (26), 59 (100). Anal. calcd for $C_{29}H_{25}N_5O_2S$ (507.61): C, 68.62; H, 4.96; N, 13.80. Found: C, 68.59; H, 4.81; N, 13.67%.

Alternative synthesis of 7k

Sodium acetate trihydrate (0.138 g, 1 mmol) was added to a solution of **8** (0.507 g, 1 mmol) in ethanol (20 mL), and the mixture was cooled to 0–5 °C in an ice bath. To the resulting cold solution was added portionwise a cold solution of benzenediazonium chloride [prepared by diazotizing aniline (0.093 mmol) dissolved in hydrochloric acid (6 M, 1 mL) with a solution of sodium nitrite (0.07 g, 1 mmol) in water (2 mL)]. After complete addition of the diazonium salt, the reaction mixture was stirred for a further 30 min in an ice bath. The solid that separated was filtered off, washed with water, and finally recrystallized from DMF to give a product that is typical in all respects (mp, mixed mp, and IR spectra) with that obtained from the reaction of **2** with **5k**.

Synthesis of 1,3-thiazine derivatives 11a–e

A mixture of thiosemicarbazone **2** (0.407 g, 1 mmol) and the appropriate arylidene- malononitrile **9a–e** (1 mmol of each) in ethanol (20 mL) containing a catalytic amount of piperidine (0.1 mL) was refluxed until all the starting material was consumed (10–12 h as monitored by TLC). The reaction mixture was then poured into acidified cold water and the precipitate was filtered, washed with methanol, and recrystallized from the appropriate solvent to give the products **11a–e**.

Ethyl 3-(1-(2-(4-amino-5-cyano-6-phenyl-6*H*-1,3-thiazin-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (11a). Yellow solid; ethanol; mp 240–242 °C; IR (KBr) ν =3423, 3276, 3159 (NH_2 and NH), 2187 (C≡N), 1691 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, J = 7.2, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.69 (s, 1H, thiazine-H), 4.03 (q, J = 7.2, 2H, CH_2), 7.19–7.37 (m, 15H, Ar-H), 8.40 (s, br, 2H, NH_2), 10.68 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ13.5, 14.0 (CH_3), 56.2 (CH), 60.2 (CH_2), 112.9, 125.5, 125.6, 125.8, 127.8, 128.1, 128.3, 128.4, 128.6, 128.9, 129.0, 129.2, 129.5, 129.6, 130.3, 138.5, 142.5, 144.1, 148.2 (Ar-C), 164.7 (C=O); MS m/z(%): 561 (M^+, 2), 386 (90), 359 (45), 128 (100), 77 (73). Anal. Calcd for $C_{31}H_{27}N_7O_2S$ (561.66): C, 66.29; H, 4.85; N, 17.46. Found C, 66.21; H, 4.68; N, 17.31%.

Ethyl 3-(1-(2-(4-amino-6-(4-chlorophenyl)-5-cyano-6*H*-1,3-thiazin-2-yl)hydrazono) ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (11b). Yellow solid; ethanol; mp 170–172 °C; IR (KBr) ν =3423, 3287, 3150 (NH_2 and NH), 2212 (C≡N), 1711 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ0.97 (t, J = 7.2, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.88 (s, 1H, thiazine-H), 4.01 (q, J = 7.2, 2H, CH_2), 7.18–7.38 (m, 14H, Ar-H), 8.39 (s, br, 2H, NH_2), 10.68 (s, br, 1H, NH); MS m/z(%): 595 (M^+, 3), 494 (54), 301 (28), 128 (100), 64 (80). Anal.Calcd for $C_{31}H_{26}ClN_7O_2S$ (596.10): C, 62.46; H, 4.40; N, 16.45. Found C, 62.38; H, 4.27; N, 16.35%.

Ethyl 3-(1-(2-(4-amino-5-cyano-6-(4-methoxyphenyl)-6*H*-1,3-thiazin-2-yl) hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (11c). Yellow solid; ethanol; mp 185–187 °C; IR (KBr) ν =3423, 3285, 3164 (NH_2 and NH), 2210 (C≡N), 1708 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ0.97 (t, J = 7.2, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 1H, thiazine-H), 4.03 (q, J = 7.2, 2H, CH_2), 6.96–7.38 (m, 14H, Ar-H), 8.41 (s, br, 2H, NH_2), 10.68 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ13.5, 14.0, 21.6 (CH_3), 43.5 (CH), 56.2 (OCH_3), 60.7 (CH_2), 110.3 114.0, 120.5, 125.5, 127.9, 128.1, 128.3, 128.9, 129.2, 129.4, 129.9, 130.4, 138.5, 142.5, 144.1, 148.2 (Ar-C), 164.7 (C=O); MS m/z(%): 591 (M^+, 4), 539 (86), 484 (94), 301 (40), 121 (64), 77 (100). Anal. Calcd for $C_{32}H_{29}N_7O_3S$ (591.68): C, 64.96; H, 4.94; N, 16.57. Found C, 64.87; H, 4.754; N, 16.46%.

Ethyl 3-(1-(2-(4-amino-5-cyano-6-(styryl)-6*H*-1,3-thiazin-2-yl)hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (11d). Yellow solid; dioxane; mp 220–222 °C; IR (KBr) ν =3423, 3277, 3159 (NH_2 and NH), 2213 (C≡N), 1691 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.03 (t, J = 7.2, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.88 (s, 1H, thiazine-H), 4.06 (q, J = 7.2, 2H, CH_2), 7.19–7.38 (m, 17H, Ar-H and –CH=CH), 8.41 (s, br, 2H, NH_2), 10.68 (s, br, 1H, NH); MS m/z(%): 587 (M^+, 2), 484 (43), 208 (52), 119 (73), 59 (100). Anal. Calcd for $C_{33}H_{29}N_7O_2S$ (587.69): C, 67.44; H, 4.97; N, 16.68. Found C, 67.37; H, 4.83; N, 16.49%.

Ethyl 3-(1-(2-(4-amino-6-(benzo[d][1,3]dioxol-5-yl)-5-cyano-6*H*-1,3-thiazin-2-yl) hydrazono) ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (11e). Yellow solid; ethanol; mp 181–183 °C; IR (KBr)

ν =3423, 3278, 3162 (NH_2 and NH), 2223 (C≡N), 1692 (C=O), 1611 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.17 (t, J = 7.2, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.86 (s, 1H, thiazine-H), 4.13 (q, J = 7.2, 2H, CH_2), 6.12 (s, 2H, OCH_2O), 7.16–7.54 (m, 13H, Ar-H), 8.34 (s, br, 2H, NH_2), 10.67 (s, br, 1H, NH); MS m/z(%): 605 (M^+, 11), 472 (39), 389 (73), 128 (100), 64 (46). Anal. Calcd for $C_{32}H_{27}N_7O_4S$ (605.67): C, 63.46; H, 4.49; N, 16.19. Found C, 63.41; H, 4.37; N, 16.06%.

Synthesis of ethyl 3-(1-((5-(2-methoxy-2-oxoethylidene)-4-oxothiazolidin-2-ylidene) hydrazono)ethyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (13). A mixture of thiosemicarbazone **2** (0.407 g, 1 mmol) and dimethyl acetylene- dicarboxylate (0.142 g, 1 mmol) in methanol (15 mL) was refluxed for 2 h. The formed precipitate was filtered, washed with methanol, and recrystallized from DMF to give product **13**. Canary yellow solid (78%); DMF; mp 226–228 °C; IR (KBr) ν =3433 (NH), 1744, 1627 (2C=O), 1591 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.06 (t, J = 7.2, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.76 (s, 3H, $COOCH_3$), 4.29 (q, J = 7.2, 2H, CH_2), 6.65 (s, 1H, C=CH), 7.27–7.42 (m, 10H, Ar-H), 12.90 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ13.8, 15.2, 52.3 (CH_3), 60.8 (CH_2), 114.4, 125.6, 127.8, 128.9, 128.4, 129.0, 129.2, 129.6, 142.8, 143.4, 158.0, 159.8 (Ar-C), 163.7, 165.5, 165.7 (C=O); MS m/z(%): 517 (M^+, 27), 471 (29), 272 (45), 200 (100), 77 (92). Anal. Calcd for $C_{26}H_{23}N_5O_5S$ (517.56): C, 60.34; H, 4.48; N, 13.53. Found C, 60.27; H, 4.40; N, 13.46%.

3.2. Pharmacology: cell culture and viruses

McCoy cells were grown in DMEM (Cultilab, BR) supplemented with 5% fetal bovine serum (FBS, Gibco BRL, USA), penicillin G (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL) (Gibco BRL). The cell culture was maintained at 37 °C in a humidified 5% CO_2 atmosphere. Stock of the fixed strain viral titration was obtained by the limiting-dilution method and expressed as 50% of the tissue culture infection dose per mL (TCID50/mL) (Reed LJ 1938). Vero (ATCC: CCL 81) and *Hep-2* (ATCC: CCL 23) cells were grown in MEM (Cultilab, BR) supplemented with 5% FBS (Gibco BRL, USA), penicillin G (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL) (Gibco BRL, USA). The cell culture was maintained at 37 °C in a humidified 5% CO_2 atmosphere. Herpes simplex virus type 1 (*HSV-1*, *KOS strain*) and *adenovirus type 5* (AdV-5) viral stocks were prepared as described by Simões et al. and the infected cells fluids were harvested, titrated, and stored at –80 °C until use. Both HSV- 1 and Adv-5 were titrated as described elsewhere.[45]

Antiviral activity is identified as confluent, relatively unaltered monolayers of stained Vero cells treated with *HSV-l.* Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by *HSV-l 126–128.* Aphidicolin (0.005 μg/mL) was used as a positive control. The compounds were tested against *HSV-1* grown on Vero.

4. Conclusion

We developed a simple and convenient method for the synthesis of new ethylidenehydrazonothiazoles and ethylidenehydrazono-1,3-thiazines incorporating pyrazole moiety. All the new products were evaluated for their antiviral activity. The results indicated that some of the products exhibited high to moderate potency against the rabies virus compared with the reference drug aphidicolin used in the investigation.

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